

Olfactory-Based Orientation in Artificially Imprinted Sea Turtles

Abstract. *Sea turtles (Lepidochelys kempii) are being artificially imprinted to Padre Island, Texas, in an effort to establish a new nesting population. These turtles spent more time per exposure in solutions made of Padre Island sand and seawater than in control solutions in a multiple-choice test. This is evidence that sea turtles may detect differences in natural water samples and remember olfactory cues to which they were exposed neonatally and that these differences may affect their orientation behavior. This suggests that imprinting could be used as a conservation technique for establishing new breeding populations of endangered sea turtles.*

Kemp's ridley, *Lepidochelys kempii*, is a rare and endangered sea turtle species (1). Almost the entire species nests on a 15-km section of beach near Rancho Nuevo, Mexico. In 1978, the United States joined Mexico in an intense conservation project attempting to establish a second nesting population of Kemp's ridleys on Padre Island, Texas, 400 km north of Rancho Nuevo.

Typically, eggs are collected at Rancho Nuevo during oviposition, placed in containers of Padre Island sand, and transported to Padre Island National Seashore where they are hatched (2). Hatchlings are released on the beach and allowed to enter the surf. This experimental conservation strategy, known as artificial "imprinting," is based on olfactory imprinting (1, 3), an unproven hypothesis suggesting a learning of the olfactory nature of the nesting beach or the

adjacent waters, or both, by the hatchlings as they leave the nest and migrate out to sea. The turtles would store this olfactory information without reexposure until many years later when they return as adults to nest. At that time, the stored olfactory information would facilitate the animals' navigation as they approach the nesting beach.

As part of the effort to save the Kemp's ridley, juveniles are raised for 9 to 12 months at the National Marine Fisheries Laboratory, Galveston, Texas. We reasoned that if these juvenile ridleys are in fact imprinted to Padre Island, they might show responses (other than homing) to solutions derived from Padre Island as part of their normal juvenile behavior. We report that 4-month-old Padre Island imprinted ridleys showed a preference for solutions made from Padre Island sand and seawater in a multiple-choice situation. The turtles can apparently detect differences in naturally occurring waters, and this sensory information affects the animal's orientation behavior. This observation suggests that imprinting to new beaches can occur and that artificial imprinting could be developed as a strategy for saving these endangered sea turtles.

Beach sand and seawater samples were collected at the imprinting site and at a site 293 km to the northeast on Galveston Island (4.2 km west of Jamaica Beach) in the summer of 1980 and refrigerated for later use. Each test involved placing a single turtle in an automated monitoring tank (4) (Fig. 1). After a 30-minute acclimation period in the tank, each turtle could choose among four compartments; these contained solutions from either Padre or Galveston

Island (5) and two untreated solutions. Seawater was continuously siphoned from a peripheral moat into the back of each compartment at 45 liters per hour, and treatment solutions were pumped into the back of compartments at 1 liter per hour. The order in which solutions were pumped into compartments was systematically varied between tests to eliminate compartment bias. Galveston solution served as one control with two compartments being untreated controls. Total entries, total time spent, and time spent per entry (6) into compartments were recorded during 4-hour monitoring periods for 12 4-month-old ridley turtles hatched in 1981.

Turtles spent significantly more time per entry (7) ($P \leq 0.05$) in water treated with Padre solution than in either that treated with Galveston solution or the untreated solutions, in which they spent approximately equal time per entry (Fig. 2). We interpret this as a preference by Padre Island imprinted Kemp's ridleys for the solution from there. This suggests that turtles may orient to solutions through a behavior not directly attributable to feeding (8).

The turtles also distinguished Galveston solution from both the Padre solution

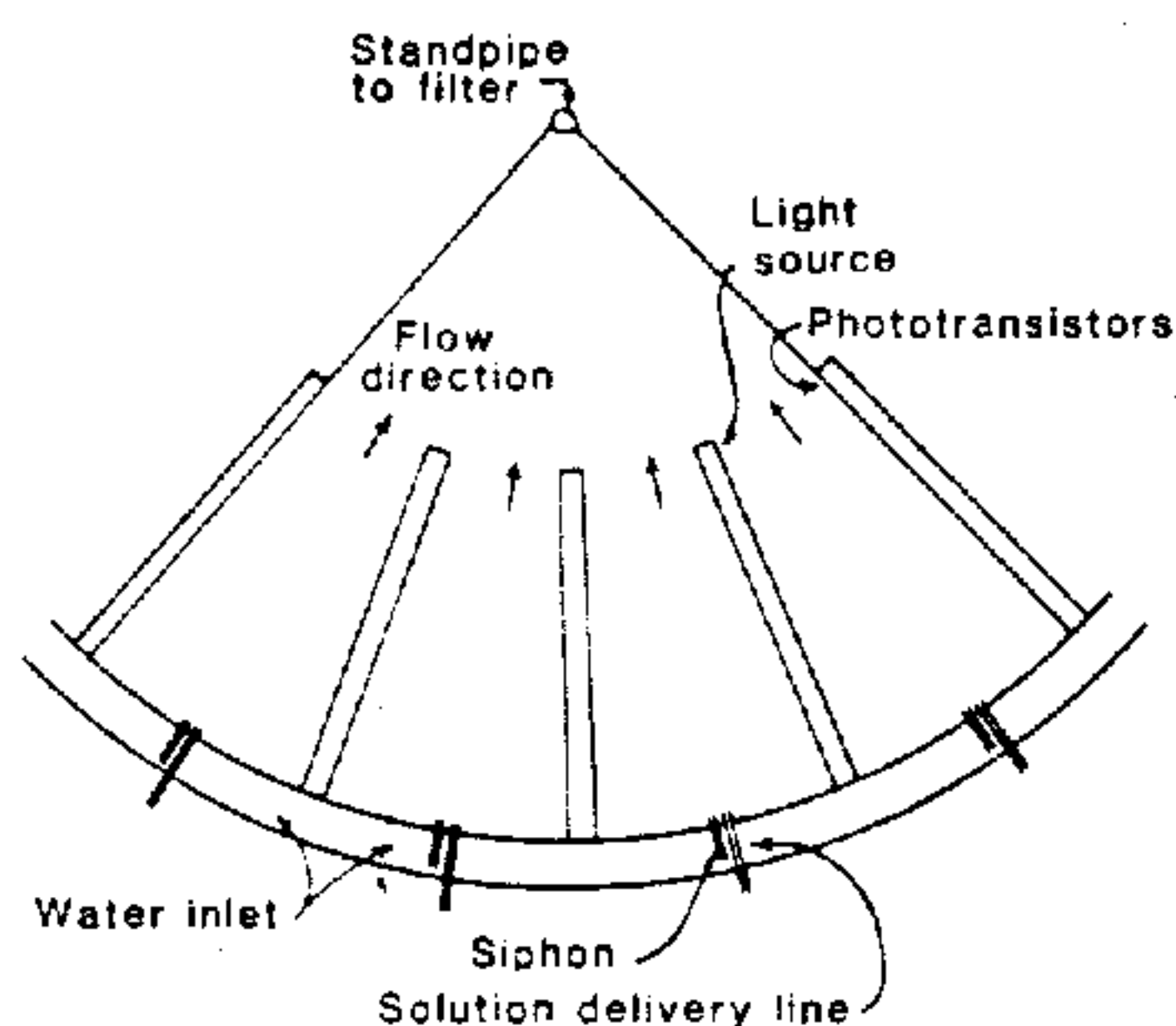


Fig. 1. Schematic diagram of automated system for monitoring marine turtle behavior (4). Solutions made of seawater that was used to wash beach sand were pumped into compartments, and the turtles' responses to these solutions were recorded.

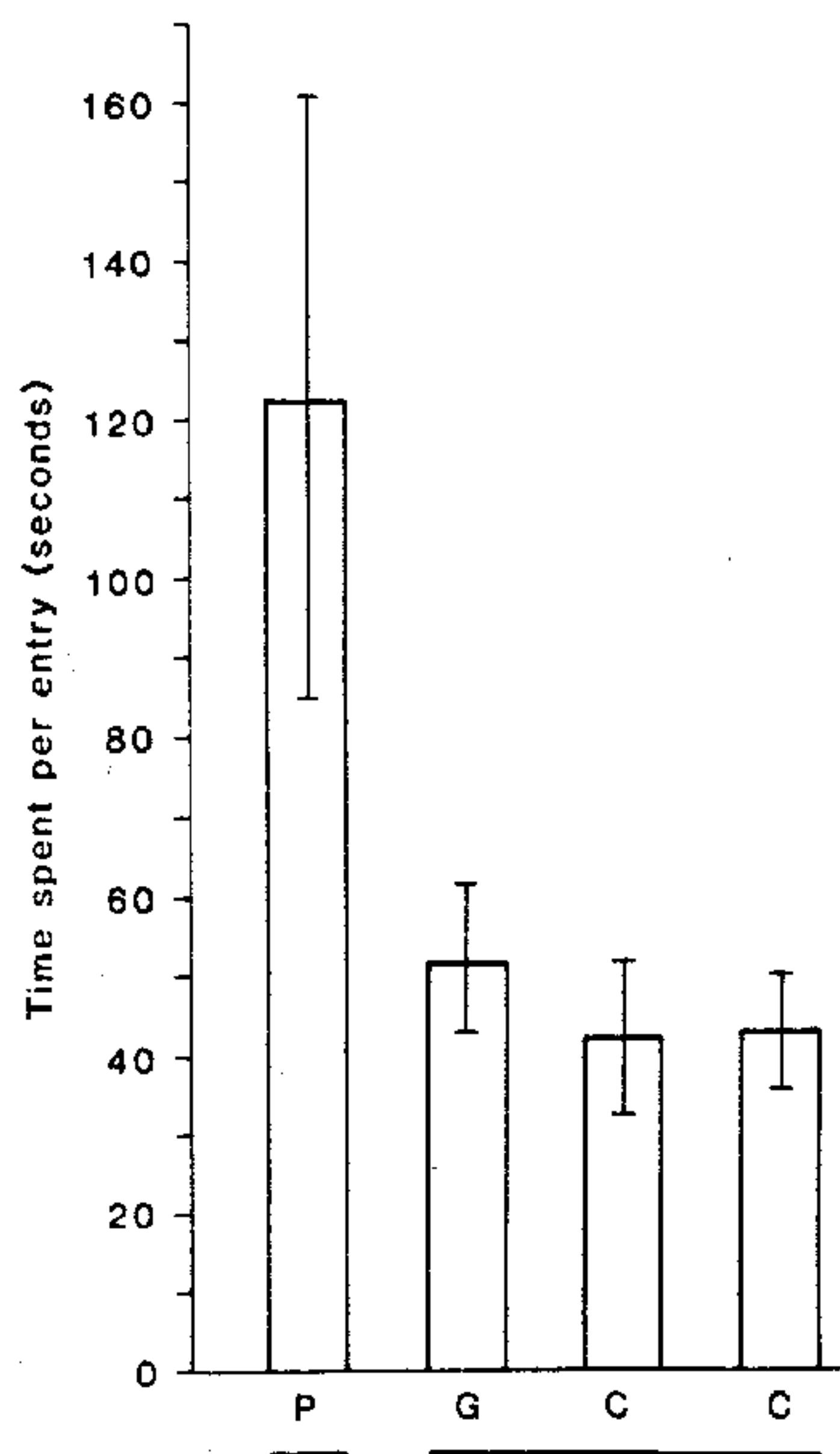


Fig. 2. Mean (\pm standard error of the mean) time spent per entry in compartments treated with Padre solution (P) ($N = 131$), Galveston solution (G) ($N = 254$), and untreated (C) ($N = 207$ and 218) compartments. Compartments underlined with the same line are not significantly different (Tukey's test, $P \leq 0.01$).

and untreated controls. Turtles entered compartments treated with Galveston solution more often ($P \leq 0.05$) than those that were untreated or treated with Padre solution (Table 1). They entered Padre solution compartments less often than untreated compartments, but the total time spent in Padre solution and Galveston solution compartments was similar. Entries in Padre solution compartments were low because turtles stayed much longer in this solution when they did enter. Total time spent in both Padre and Galveston solution compartments was greater but not statistically different than that for untreated compartments. When turtles entered Galveston solution compartments they did not stay there longer than if they had entered untreated compartments. We interpret the high number of entries in Galveston solution to be a result of the animals' unrewarded appetitive behavior or search for a releasing stimulus (9). We suggest that the high amount of time spent per entry in Padre solution indicates a rewarded appetitive behavior or drive-reducing consummatory act.

From the behavior exhibited by these ridley turtles, one might predict that they would move (migrate?) in the direction of Padre Island when they detect olfactants emanating from the artificial imprinting area. In our test situation, however, we suggest that turtles accepted Padre solution as a substitute for a more appropriate releasing stimulus such as food, possibly because they were imprinted to Padre Island. In nature one would expect the threshold of the migratory drive to the nesting beach to be higher than thresholds for an appropriate juvenile behavior such as feeding. This would result in juvenile behaviors having priority over migratory behavior. In reproductive adults, one might predict that the threshold of the migratory drive would be lowered. The fact that nesting beaches are often far from feeding areas suggests that migration becomes a higher priority than feeding in reproductive turtles. Also, the observation that estrogen-treated turtles eat less suggests that these threshold changes have a physiological basis (10).

Our results suggest that Kemp's ridley turtles, artificially imprinted to Padre Island, are behaviorally imprinted to the chemosensory environment of Padre Island. This is the only experimental evidence that sea turtles might imprint and that artificial imprinting may be a valid conservation practice. The best proof for imprinting would be the establishment of a new population by artificially imprinting hatchlings to a novel beach. Such

Table 1. Mean (\pm standard error of the mean) total entries and time spent in Padre solution (P), Galveston solution (G), and untreated (C) compartments for the 12 turtles.

Com-part-ment	Number of entries	Time spent (seconds)
P	10.89 \pm 2.84	1290.0 \pm 474.0
G	21.2 \pm 8.32	1176.0 \pm 336.0
C	17.25 \pm 8.38	642.0 \pm 300.0
C	18.2 \pm 10.46	756.0 \pm 426.0

phenomena as long times to maturity (15 to 45 years in *Chelonia mydas*), temperature-dependent sex differentiation, and nearly insurmountable tagging limitations were not anticipated when this work started in the 1950's (1, 4). Thus, laboratory experimentation to evaluate the imprinting question may be the only viable approach to the problem. In fact, failure to establish new nesting populations through artificial imprinting techniques would not disprove the possibility that turtles imprint to their natal beach. Artificial imprinting techniques currently used for conservation may be inappropriate or incomplete to behaviorally imprint turtles. Until more is known about the timing and stimulus requirements of a possible imprinting mechanism, the methods used to artificially imprint turtles must be considered to be experimental.

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References and Notes

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2. E. F. Klima and J. P. McVey, in *Biology and Conservation of Sea Turtles*, K. A. Bjorndal, Ed. (Smithsonian Institution Press, Washington, D.C., 1981), p. 481.
3. A. F. Carr, *NASA, Spec. Publ.* 262 (1972), p. 469.
4. D. W. Owens, M. A. Grassman, J. R. Hendrickson, *Herpetologica* 38, 124 (1982).
5. Solutions to be pumped into the behavior monitoring tank (4) (Fig. 1) were prepared from samples by washing 1 kg of sand with 1 liter of seawater. A sufficient volume of seawater was then added to bring the total volume to 4 liters. Equal volume (1143 ml) of synthetic seawater at concentrations appropriate to equilibrate the salinity of the solutions to each other and the monitoring tank water were then added to facilitate mixing. Because equal volumes of water were added to each sample, concentrations of possible olfactants in the two samples did not change in relation to each other.
6. In a previous experiment only total entries and total time spent in compartments were reported (4). Because entries and time spent were not independent variables in our investigations, it was important not only to consider these two variables, but also to consider the length of time an animal spent each time it entered a compartment.
7. R. R. Sokal and F. J. Rohlf, *Biometry* (Freeman, San Francisco, 1981); total entries and total time spent were analyzed with a two-factor (compartment by solution) log likelihood ratio test. This test was designed for analyzing discrete variables. Because time was continuous, total time spent was transformed by dividing by 600 seconds. This was established by a runs test of independence to be a discrete unit of time in the animals' response. A nested-factorial analysis of variance on data on time spent per entry was used to account for individual and compartment variation. There was a significant treatment-solution effect [$F(3) = 2.64$, $P \leq 0.05$]. Also, data on time spent per entry were further analyzed with a Tukey's test ($P \leq 0.01$) on treatment-solution means. Data on time spent per entry underwent arcsin transformations to meet the homogeneity assumption of analysis of variance.
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11. We thank J. Matis for statistical assistance and J. Dixon and C. T. Fontaine for comments on the manuscript. This research was funded in part by the Texas A&M University Sea Grant College Program under grant 4A79AA-D-00127. This research was conducted under U.S. Fish and Wildlife Service permits PRT2-4481 and PRT2-1770 and permit 286 from the Texas Parks and Wildlife Department. Turtles were donated by Mexico to the Kemp's ridley recovery project under permit 242.2-2610. This investigation is a contribution of the joint Mexico-United States Kemp's ridley recovery program through the National Marine Fisheries Service, Galveston Laboratory.

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